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Drinking Water Criteria Document for Carbofuran (Final Draft)

Life Systems, Inc., Cleveland, OH

Prepared for

Environmental Protection Agency, Washington, DC

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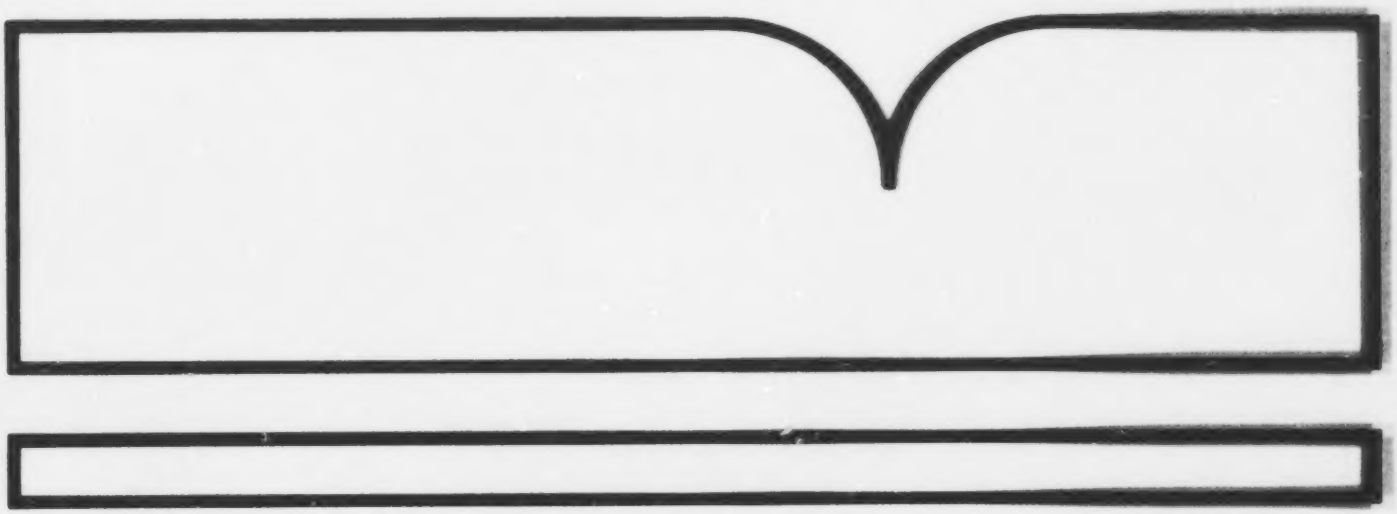

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FINAL DRAFT FOR THE DRINKING WATER
CRITERIA DOCUMENT ON CARBOFURAN

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Confidential Business Information (CBI) from EPA files for this chemical has been reviewed, and non-CBI summaries of key health effects information are included in this document. Personnel involved with the revising and editing of this document have been instructed in the proper safeguards for maintaining the confidentiality of this information.

To adequately safeguard the CBI aspects of this information, the non-CBI summaries generally contain less detail on experimental design, individual study results, etc., than do those studies that have been cited from the open literature. Thus, some variation in the writing style, level of detail and ability of the reader to make independent interpretations of the cited studies was unavoidable.

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16. ABSTRACT

The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on carbofuran. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of carbofuran
- Toxicokinetics and human exposure to carbofuran
- Health Effects of carbofuran in humans and animals
- Mechanisms of toxicity of carbofuran
- Quantification of toxicological effects of carbofuran

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I. SUMMARY

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is a carbamate pesticide introduced into agricultural use in the United States in 1968. It is used primarily on corn (soil application) and alfalfa (foliar application). Pure carbofuran is a white, crystalline solid with a water solubility of 700 ppm. It is formulated as 2%, 3%, 5% and 10% granules and a 48% flowable paste. Soil applications vary between 0.25 and 1 kg carbofuran/hectare, while foliar applications are 0.5 to 4 kg carbofuran/hectare.

Carbofuran is degraded in the environment both chemically and biologically by oxidative and hydrolytic mechanisms. Oxidative reactions lead to two important metabolites, 3-hydroxycarbofuran and 3-ketocarbofuran. Both of these metabolites have toxicological properties comparable to those of the parent compound. Hydrolytic degradation results primarily in the removal of the methylcarbamyl moiety, giving carbofuran phenol (2,3-dihydro-2,2-dimethyl-7-benzofuranol). Structurally analogous phenolic metabolites are formed by hydrolysis of 3-hydroxycarbofuran and 3-ketocarbofuran. The toxicological result of such metabolism is loss of antiacetylcholinesterase properties, which essentially detoxifies the molecule.

The absorption, distribution and excretion of carbofuran in mammals are typical of those of other carbamate insecticides. Carbofuran is absorbed rapidly. Rats absorb 63.4% of an oral dose from the stomach in 30 minutes

and 71.7% of a dermal dose within 15 minutes. Distribution to organs is rapid with excretion of 70% of an oral dose in urine and 5% in feces in the first 24 hours.

Carbofuran is an acetylcholinesterase-inhibiting insecticide and the toxic symptoms in animals are typical of that mode of action. The acute oral LD_{50} values in mammals range from 2.0 to 14.1 mg/kg. Lethal effects following acute exposure are the result of inhibition of acetylcholinesterase. Other acute effects on the immune system and the blood have been reported. These effects, however, are not lethal and appear to be readily reversible when exposure to the compound is terminated.

Inhibition of acetylcholinesterase is the most sensitive indicator recognized of the toxic effects of carbofuran. A 2.5-mg/kg dose administered orally to Sprague-Dawley rats resulted in definite symptoms of acute poisoning. An oral dose of 0.05 mg/kg resulted in only minimal effects on blood and liver acetylcholinesterase activity and no statistically significant effects on brain cholinesterase. An oral dose of 0.25 mg/kg resulted in minimal but statistically significant effects on brain cholinesterase. In chronic feeding studies, the No-Observed-Adverse-Effect Levels (NOAELs) were 0.8 mg/kg/day for rats and 3.6 mg/kg/day for mice, based on inhibition of acetylcholinesterase.

There have been many short-term assays of the mutagenic potential of carbofuran. There are a few positive results, particularly in the Ames test with Salmonella typhimurium strains TA98 and TA1538. Tests with other Salmonella strains and other short-term tests were negative. Carcinogenicity bioassays with rats and mice in two-year feeding studies resulted in no increase in tumor incidence in either species.

It is possible to prepare N-nitrosocarbofuran chemically by allowing carbofuran to react with nitrite in an acidic medium. Several in vitro studies have demonstrated the feasibility of this reaction, and tests of N-nitrosocarbofuran and the nitroso derivatives of 3-hydroxycarbofuran and 3-ketocarbofuran in Salmonella typhimurium strain TA100 gave positive mutagenic results. These materials are among the most mutagenic compounds tested in the TA100 strain. N-Nitrosocarbofuran administered to rats resulted in increased numbers of stomach tumors. Of particular concern is the suggestion that the pH of the human stomach would favor the formation of N-nitrosocarbofuran, but there is only limited evidence that this reaction occurs in vivo.

The reproductive and teratogenic effects of carbofuran were assessed in beagle dogs, rats and rabbits. Some testicular degeneration was noted in beagles administered approximately 12.5 mg/kg/day carbofuran in the diet. In rats and rabbits, no-effect levels were 1.0 and 2.0 mg/kg/day, respectively. No terata were observed in any of these species.

In humans, dermal and oral exposure to carbofuran results in symptoms of cholinesterase depression, including nausea, stomach cramps, vomiting, increased salivation and tremors. In a controlled human experiment, single oral doses of carbofuran above 0.05 mg/kg/day resulted in symptoms of acetylcholinesterase inhibition.

The mechanism of action of carbofuran is that of a potent inhibitor of acetylcholinesterase. Carbofuran is a direct inhibitor, not requiring metabolic alteration to become active.

A No-Observed-Adverse-Effect Level (NOAEL) of 0.05 mg/kg/day, based on absence of symptoms of acetylcholinesterase inhibition in humans, was used to calculate a one-day Health Advisory (HA) for the 10-kg child of 0.05 mg/L. A one-day HA for the 70-kg adult was calculated as 0.18 mg/L. The same values were used for the ten-day HA values since effects on acetylcholinesterase are reversible and do not appear to be cumulative. An Adjusted Acceptable Daily Intake (AADI) of 0.18 mg/L was calculated, using a NOAEL of 0.50 mg/kg/day from a one-year feeding study in dogs.

No prior guidelines or standards governing oral exposure to carbofuran were located. For inhalation exposure in the workplace, the time-weighted (8 h/day) threshold limit value is 0.1 mg/m^3.

II. PHYSICAL AND CHEMICAL PROPERTIES

Carbofuran is a broad-spectrum carbamate insecticide, acaricide and nematocide. Carbofuran formulations have been used widely in efforts to control corn rootworm, rice water weevils and mosquitoes, sugarcane nematodes and wireworms, as well as alfalfa weevil on alfalfa and thrips and nematodes on peanuts (Cook 1973). Although carbofuran is registered for use on many crops, its major use (91%) is in corn production, while 9% is used on alfalfa and rice (based on 1974 data). It is registered as a contact insecticide for crops and as a systemic for soil treatment (Midwest Research Institute 1976). The first registered use for carbofuran in the U.S. was on field corn in 1968. The use of chlorinated hydrocarbons in the U.S. has decreased since the early 1970s while other less persistent pesticides, including carbofuran, have been used more frequently. Domestic usage in 1972 was estimated by the Midwest Research Institute (1976) as 6.0 million pounds. By 1974 it was estimated that 6.8 million pounds was used on corn in the U.S. alone (Midwest Research Institute 1976). Although total use figures are not available, over 7 million pounds of carbofuran was used in 1974.

Synonyms for carbofuran include Furadan,[a] Curaterr,[a] Bay 70143, NIA 10242 and ENT 27164. Physical and chemical properties for carbofuran (chemical name, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate) are listed in Table II-1. In addition to the reported water solubility of

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[a]Registered trademark.

Table II-1 Physical and Chemical Properties of Carbofuran

Property	Value
Chemical Abstracts Registry No.	1553-66-2
Synonyms	Furadan,[a] Curaterr,[a] Bay 70143, NIA 10242 ENT 27164
Chemical Name	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate
Wiswesser Line Notation	T56 BOT&J C1 C1 IOVM1
Chemical Formula	$C_{12}H_{15}NO_3$
Molecular Weight	221.26
Elemental Analysis	C, 65.14%; H, 6.83%; N, 6.33%; O, 21.69%
Physical State	White, crystalline solid
Odor	Odorless, slightly phenolic
Flammability	Not flammable; will support combustion if ignited
Explosive Hazard	None
Corrosive Action	None
Melting Point	153° to 154°C
Density	1.180 (20°C)
Vapor Pressure	2×10^{-5} mm Hg (33°C) 1.1×10^{-4} mm Hg (50°C)
Solubility % weight/weight, 25°C	
Acetone	15
Acetonitrile	14
Benzene	4
Cyclohexanone	9

continued-

[a]Registered trademark.

Table II-1 - continued

Property	Value
Dimethyl formamide	27
Dimethyl sulfoxide	25
Ethanol	4
Kerosene	<1
N-Methyl-2 pyrrolidone	30
Methyl chloride	12
Petroleum ether	<1
Xylene	<1
Water	0.07 (700 ppm)

Adapted from Windholz (1983), Kuhr and Dorough (1976), Midwest Research Institute (1976), Cook (1973).

700 mg/L at 25°C, Bowman and Sans (1979) determined a solubility of 320 mg/L at 19°C .

Carbofuran is prepared by thermal rearrangement and cyclization of 1-methallyloxy-2-nitrobenzene to 2,3-dihydro-2,2-dimethyl-7-nitrobenzofuran, which is reduced to the amine, diazotized and converted to 2,3-dihydro-2,2-dimethylbenzofuran-7-ol. This product is converted to the methyl carbamate by reaction with methyl isocyanate in the presence of triethylamine (Martin and Worthing 1977). Carbofuran is formulated as 2%, 3%, 5% or 10% granules and as a 48% flowable paste. Furadan[a] is usually applied to foliage at 0.25 to 2 kg active ingredient/hectare, placed in or above seed furrows at 0.5 to 4 kg/hectare or broadcast at rates of 6 to 10 kg/hectare (Martin and Worthing 1977).

Carbofuran may undergo a number of chemical reactions, including photodecomposition, oxidation and hydrolysis (Midwest Research Institute 1976). The structures and names of carbofuran and its main products are given in Figure II-1. Cook (1973) stated that carbofuran hydrolyzes under alkaline or strongly acidic conditions, with cleavage at the carbamate linkage, but that hydrolysis does not occur in neutral or slightly acidic solutions. These statements are confirmed by the studies of Metcalf et al. (1968).

A matter of considerable environmental interest is the possibility of formation of N-nitroso derivatives of the N-methylcarbamate insecticides. The original work on this reaction utilized carbaryl, the most extensively used

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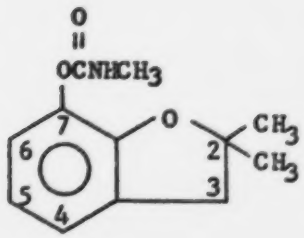
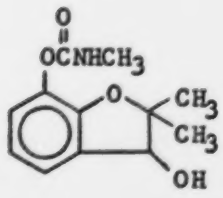
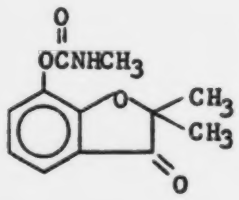
[a]Registered trademark.



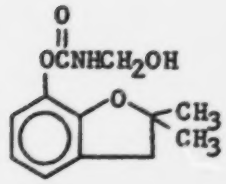
Carbofuran



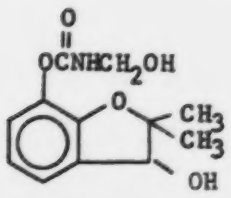
3-Hydroxycarbofuran



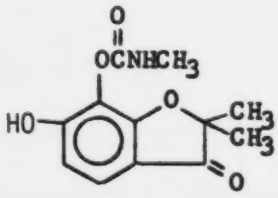
3-Ketocarbofuran



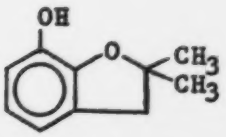
N-Hydroxymethyl carbofuran



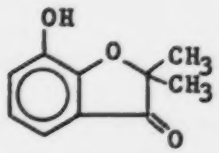
3-Hydroxy-N-hydroxy-methyl carbofuran



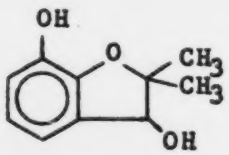
3-Keto-6-hydroxy-carbofuran



Carbofuran phenol (2,2-dimethyl-7-hydroxy-2,3-dihydrobenzofuran) (2,3-dihydro-2,2-dimethyl-7-benzofuranol)



3-Ketocarbofuran phenol



3-Hydroxycarbofuran phenol

Adapted from Midwest Research Institute (1976)

Figure II-1 Chemical Structures of Carbofuran and Its Degradation Products

carbamate insecticide. Elespuru and Lijinsky (1973) demonstrated that carbaryl (0.5 or 0.1 M) plus nitrite (2.0 or 0.4 M) in aqueous solution containing 25% dimethyl sulfoxide reacted under acid conditions (pH 2.8 to 3.9) at 37°C to give yields of 5% to 11% nitrosocarbaryl after one hour. Similarly, Eisenbrand et al. (1975) produced nitrosocarbaryl by incubation of carbaryl with a five-fold molar excess of nitrite for 15 to 60 minutes in 0.1 N HCl (pH 1). Decreases in pH slightly increased the yield to a maximum of 3.2% in 1 N HCl.

Carbofuran undergoes a similar reaction to form N-nitrosocarbofuran (Seiler 1977, Lijinsky and Schmahl 1978, Nelson et al. 1980, 1981). The effect of pH on the formation (by nitrosation), stability (in vivo and in vitro) and degradative products of N-nitrosocarbofuran was observed by Rickard et al. (1982). The efficiency of nitrosation of carbofuran by nitrite in aqueous HCl solutions varied profoundly with moderate changes in pH. Reactions between carbofuran (0.5 μmol) and $NaNO_2$ (1.16 mmol, in one mL HCl solution) were allowed to proceed for ten minutes in the dark at 37°C. At pH 1 the yield of nitrosocarbofuran was 41.9%, at pH 2 the yield was down to 0.9%, and by pH 3 it had decreased to 0.01%. The authors suggested that these results indicated that the human stomach pH of 1 to 2 favors nitrosation much more heavily than does the rat stomach pH of 3 to 4.

Rickard et al. (1982) also investigated the stability of nitroso-carbofuran in buffered aqueous systems. At 37°C, nitrosocarbofuran was most stable between pH 3 (half-life of 470 minutes) and pH 5 (359 minutes) and much

less so at pH 7 (70 minutes) or pH 1.5 (34 minutes). After two hours at pH 1.5, breakdown by denitrosation was heavily favored (62.9% carbofuran), but at pH 7 the major route was hydrolysis to the carbofuranphenol (31%). Two additional unidentified products (totaling 30.8%) were observed in the pH 7 sample. Breakdown at pH 3 or 5 led to similar quantities of carbofuran and carbofuran-phenol.

III. TOXICOKINETICS

A. Absorption

Ahdaya et al. (1981) studied the absorption and distribution of radioactive ^{14}C-labeled carbofuran (1 μCi per 1 mg/kg dose), which was administered at 1 mg/kg in 0.1 mL carrier (Emulphor:ethanol:water, 1:1:8) via stomach feeding needle to fasted female ICR mice (seven to eight weeks old, 25 to 30 g). The mice were sacrificed at intervals of 1, 5, 15, 30 and 60 minutes after administration (three mice per interval), and the amount of unabsorbed radioactivity in the gastrointestinal tract was determined. Also, absorbed radioactivity was determined for selected organs by scintillation counting. The results, shown in Table III-1, indicated that 51.2% of the radioactivity was absorbed by 15 minutes and 67.4% by 60 minutes.

Ahdaya and Guthrie (1982) studied the stomach absorption of carbofuran using the same procedures as Ahdaya et al. (1981), but the stomachs of the mice were surgically ligated at the pylorus. Approximately 19% of the radioactive carbofuran was absorbed within one hour. Thus, the stomach alone is a significant route of absorption, at least when ligated. Compared to the gastrointestinal absorption obtained by Ahdaya (1981), stomach absorption was about 28% of total absorption. The amounts of carbofuran recovered from the intestine (2.6%) must have been through intestinal secretion, since the pylorus was ligated.

Table III-1 Absorption and Recovery of Radioactive Labeled Oral Doses of Carbofuran Intubated in Mouse Stomachs

Time	% Absorption[a]	% Administered radioactivity recovered	
		CO_2	Urine
1 min	34.5	-	-
5 min	44.7	-	-
15 min	51.2	1.1	5.6
30 min	63.4	3.0	17.1
60 min	67.4	6.0	24.0

[a]Absorption was calculated as the quantity: (100% - % in gastrointestinal tract).

Adapted from Ahdaya et al. (1981).

Shah et al. (1981) studied the dermal absorption of carbofuran in seven- to eight-week-old female ICR mice. Doses of ^{14}C-labeled (1 μCi per 1 mg/kg dose) carbofuran were applied in 0.1 mL acetone to a 1-cm^2 shaved area of the upper shoulder skin of the mice. Mice were sacrificed at 1, 5, 15, 60 and 480 minutes after application. Absorption, as measured by loss of radioactivity from the skin at the site of application, was determined by scintillation counting. The results (Table III-2) show that about 33% was absorbed by five minutes, 76% by 60 minutes and 95% was absorbed by 480 minutes after administration.

B. Distribution

In a study in which mouse stomachs were ligated at the pylorus prior to administration of ^{14}C-labeled carbofuran (Ahdaya and Guthrie 1982), distribution to various organs and tissues was monitored. The distribution to organs was related to stomach absorption, in this instance, since the stomach was ligated. The results are shown in Table III-3.

Shah et al. (1981) examined the time course of distribution of ^{14}C-labeled carbofuran to organs and tissues following dermal exposure. Results are shown in Table III-4. Immediately apparent in comparing Tables III-3 and III-4 is the very rapid entry of carbofuran into the organism and its subsequent distribution in blood to other organs. The distribution in organs and tissues after 480 minutes (dermal exposure) is shown in Table III-5.

Table III-2 Dermal Penetration After Topical Application of ^{14}C-Carbofuran in Mice

Time (min)	% Penetration[a]
1	28.5
5	32.6
15	71.7
60	76.1
480	94.7

[a]Values expressed as geometric mean of three measurements.

Adapted from Shah et al. (1981).

Table III-3 ^{14}C-Carbofuran Recovered in Organs and Tissues Following Absorption Through the Ligated Stomach of Mice

Time	Liver	Blood	Stomach	Intestine	Urine	Carcass
15 min	0.4[a]	0.4	2.8	1.0	0.6	3.8
30 min	0.7	0.6	4.1	2.2	2.7	4.7
60 min	0.9	1.2	4.0	2.6	5.3	4.9

[a]Values are % of total absorbed dose.

Adapted from Ahdaya and Guthrie (1982).

Table III-4 Distribution of ^{14}C-Labeled Carbofuran in Organs and Tissues Following Dermal Absorption in Mice

Organ/ Tissue	Time after administration		
	5 min	15 min	60 min
Liver	0.1[a]	2.4	0.6
Blood	<0.1	1.7	0.5
Fat	<0.1	0.2	<0.1
Excretory Products[b]	0.1	0.4	8.3
Carcass[c]	32.4	67.3	66.4

[a]Values expressed as % ^{14}C recovered, geometric mean of samples.
[b]Includes urine, CO_2 and feces.
[c]Carcass = portion of body remaining after removal of tissues and organs, as detailed in Table III-5.

Adapted from Shah et al. (1981).

Table III-5 Geometric Mean of Percent Recovered ^{14}C, 480 Minutes After Topical Application of ^{14}C-Carbofuran to Mouse Skin[a]

Organ/Tissue	%
Heart	0.1
Lungs	0.1
Brain	0.1
Kidney	0.3
Bladder	<0.1
Fat	0.2
Ear	0.3
Spleen	<0.1
Bone Marrow	0.3
Muscles	0.6
Stomach	3.3
Intestine	2.6
Liver	1.0
Blood	0.8
Excretory Products	72.8[b]
Carcass[c]	11.9

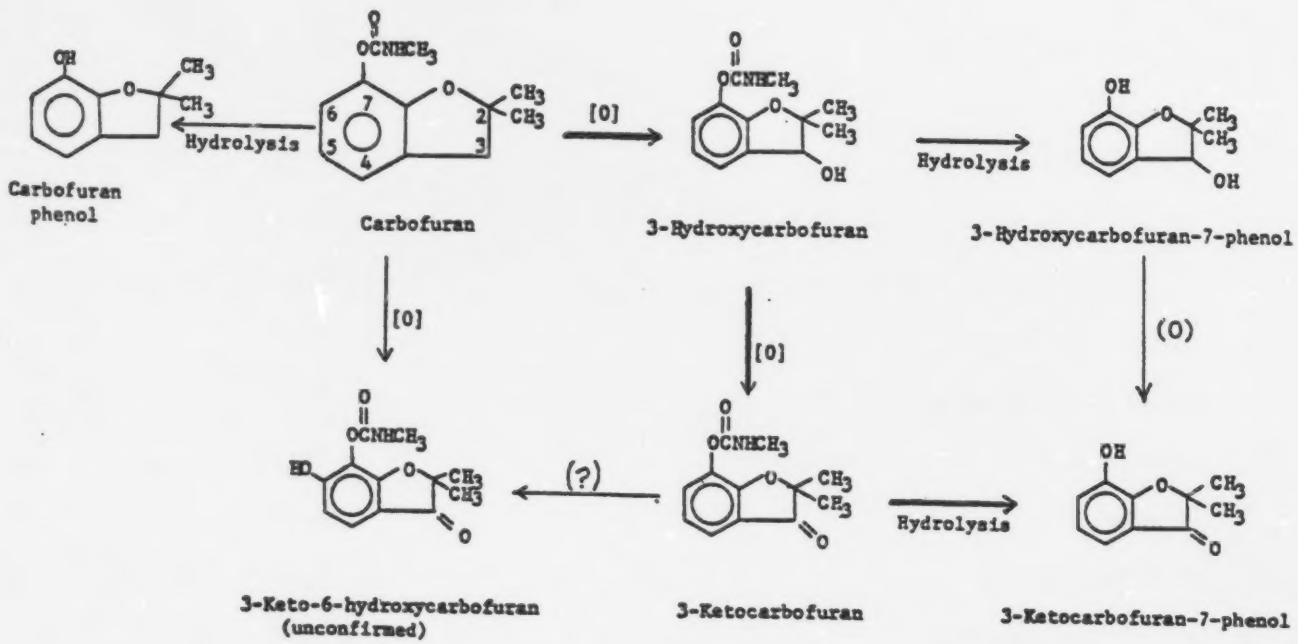
[a]A dose of 1 mg ^{14}C-carbofuran/kg was applied in acetone solution to shaved skin (upper shoulder) of ICR mice.
[b]Approximately 1/3 urine, 2/3 feces.
[c]Carcass = portion of body remaining after removal of tissues and organs listed.

Adapted from Shah et al. (1981).

C. Metabolism

The main pathway of metabolism of carbofuran in plants, insects, rats and mice appears to consist of hydroxylation at the benzylic carbon to give 3-hydroxycarbofuran (see Figure III-1). Further oxidation gives 3-ketocarbofuran, after which hydrolysis and conjugation occur. Carbofuran can be hydrolyzed to carbofuran phenol or hydrolysis can follow oxidation to yield 3-hydroxycarbofuran phenol and 3-ketocarbofuran phenol (Dorough 1968, Metcalf et al. 1968, Knaak 1971, Midwest Research Institute 1976). Hydrolysis is a significant pathway in mammals, but is minor in insects and plants.

Dorough (1968) studied the metabolites of carbofuran formed after incubation with rat liver homogenate 15,000 x g supernatant fraction. The results are shown in Table III-6. The main metabolites identified were, in order of recovery, 3-hydroxycarbofuran, N-hydroxymethylcarbofuran, carbofuran phenol, 3-hydroxy-N-hydroxymethylcarbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran phenol and 3-ketocarbofuran phenol. The 3-hydroxy-N-hydroxymethylcarbofuran, when incubated with the rat liver homogenate 15,000 x g supernatant fraction, was converted mainly to water-soluble conjugates, presumably by initial hydrolysis of the carbamyl group. Carbofuran phenol also was metabolized by the rat liver enzymes to conjugated water-soluble compounds. N-Hydroxymethylcarbofuran was metabolized to 23.9% water-soluble materials, 6.9% 3-hydroxy-N-hydroxymethylcarbofuran, 4.1% 3-hydroxycarbofuran phenol and 2.2% unidentified compounds. The 3-hydroxycarbofuran was metabolized to 23.4% water-soluble materials, 3.9% 3-hydroxycarbofuran phenol, 8.9% 3-hydroxy-N-hydroxymethylcarbo-



Adapted from Midwest Research Institute (1976) and Metcalf et al. (1968)

Figure III-1 Oxidation and Hydrolysis of Carbofuran

Table III-6 Metabolism of ^{14}C-Labeled Carbofuran by Rat Liver Homogenate 15,000 x g Supernatant Fraction

Metabolite	Yield (% Radioactivity added)
3-Hydroxy-N-hydroxymethylcarbofuran	2.07
N-Hydroxymethylcarbofuran	7.05
3-Hydroxycarbofuran	20.13
3-Ketocarbofuran	0.22
Carbofuran	58.50
3-Hydroxycarbofuran phenol	0.02
3-Ketocarbofuran phenol	0.02
Carbofuran phenol	3.82
Water soluble radioactivity	7.01
Unknown I	0.10
Unknown II	0.09
Unknown III	0.03
Total Recovery	99.06

Adapted from Dorough (1968).

furan and 0.6% unidentified compounds. Those same metabolites also were detected in the urine of rats treated orally with ^{14}C-carbofuran, mostly in the form of conjugates.

Dorough (1983) studied the metabolism of 3-hydroxycarbofuran glucoside in rats. The residual life of 3-hydroxycarbofuran in rats was longer when administered as a glucoside conjugate than when given as the free metabolite. However, it was demonstrated that the further metabolism of the glucoside and the free metabolite yielded the same metabolites in bile, although metabolism of the glucoside was slower. It was evident that the glucoside was cleaved to 3-hydroxycarbofuran, which then was metabolized further, including production of 3-hydroxycarbofuran glucuronide.

Rickard and Dorough (1984) investigated nitrosocarbofuran formation in the stomach of rats and guinea pigs in vivo. Female Sprague-Dawley rats (200 to 250 g) or Hartley guinea pigs (200 to 250 g) were given 0.50 µmol ^{14}C-labeled carbofuran in 0.1 mL ethanol by stomach tube, followed immediately by 1.16 mmol sodium nitrite in 0.3 mL water. After 15 minutes, the contents of the stomach were removed, extracted with ether, fractionated by two-dimensional thin-layer chromatography and ^{14}C-nitrosocarbofuran was quantitated by direct liquid scintillation of the gel. Only low levels of nitrosocarbofuran were detected: 0.65% in guinea pig stomach and 0.03% in rat stomach. The authors indicated that recovery of nitrosocarbofuran was only about 50% during the extraction procedure, but that the low yields were probably related to poor mixing of reactants in the stomach as well as

absorption of reactants and/or the products. Similar results were obtained when carbofuran and sodium nitrite were given in feed. Higher yields were obtained when carbofuran and sodium nitrite were mixed with gastric contents in vitro: 30% nitrosation with guinea pig and 0.5% with rat stomach contents. The difference was attributed to the pH of the gastric contents (pH 1 to 2 for the guinea pig and pH 3 to 5 for the rat). Since the pH in human stomach is also about 1 to 2, the authors concluded that nitrosation of carbofuran in humans would be similar to that in guinea pigs.

N-Nitrosocarbofuran was administered to rats by stomach tube, followed by immediate sacrifice and recovery of labeled material (Rickard et al. 1982). The major form recovered was nitrosocarbofuran (42.9%). Substantial hydrolysis (14.5% carbofuran phenol) was observed, while denitrosation (0.8% carbofuran) was only a minor route of breakdown. Some material (3.6%) was water soluble, and 24.1% was unextracted by ether, giving a total recovery of 89.3% of the administered dose.

D. Excretion

Dorough (1968) investigated the elimination of ring ^{14}C-labeled carbofuran after oral administration of a 4.0-mg/kg dose to 200-g rats (sex and strain not specified). The amounts found in urine and feces at succeeding time intervals after treatment are shown in Table III-7. Excretion of carbofuran was rapid, with 74.5% being eliminated in the first 24 hours after administration. Hurst and Dorough (1978) showed that the half-time for elimination for carbofuran increased linearly with increased dosage in rats.

Table III-7 Elimination of Radioactivity from Rats Treated Orally With Ring ^{14}C-Labeled Carbofuran

Hours after treatment	Cumulative % of administered dose	
	Urine	Feces
2	5.9	0.3
4	15.0	0.5
6	21.4	0.5
12	37.8	0.9
24	72.2	2.3
32	87.7	2.4
48	89.0	2.5
56	89.7	3.0
72	90.8	3.3[a]
96	91.4	--
120	91.6[a]	--
	Total:	94.9%

[a]Last sample containing radioactivity.

Adapted from Dorough (1968).

In their study of ^{14}C-labeled carbofuran administered to fasted mice via stomach feeding needle, Ahdaya et al. (1981) reported that after 60 minutes, 6% and 24% of the radioactively labeled doses were observed in CO_2 and urine, respectively (see Table III-1).

Excretion also was examined in the study by Shah et al. (1981) of the distribution of ^{14}C-labeled carbofuran in young female mice. Eight hours after dermal administration, 72.8% was excreted, approximately two thirds in feces and one third in urine. Thus, 48.7% of the total dose applied to the animals was excreted in feces (67% x 72.8%).

Marshall and Dorough (1984) investigated the excretion of 3-hydroxycarbofuran, one of the major metabolites of carbofuran. Ring-labeled ^{14}C-3-hydroxycarbofuran was administered by intubation to male Sprague-Dawley rats. After 1.5 hours, 40% of the label was exreted in urine and at 6 hours, 79% had been excreted in urine. Both glucuronide and sulfate conjugates were major components of the urinary radiolabeled material, indicating that biliary metabolism was important in the excretory pathway.

E. Bioaccumulation and Retention

No data regarding tissue levels as a function of time in a long-term exposure study were found in the literature. However, the data summarized in Section C (Metabolism) indicate that carbofuran is metabolized rapidly in the

body, and the data summarized in Section D (Excretion) indicate that carbofuran is excreted efficiently from the body in feces, urine and expired air. Yu et al. (1974) showed that carbofuran does not accumulate in terrestrial or aquatic organisms in a model ecosystem.

F. Summary

The absorption, distribution and excretion of carbofuran are typical of those of other carbamate insecticides. Carbofuran is rapidly absorbed. In rats, 67.4% of an oral dose and 76% of a dermal dose were absorbed in 60 minutes. Distribution to various tissues and organs is rapid. Carbofuran is metabolized by oxidative and hydrolytic pathways to yield metabolites which undergo conjugation to form water-soluble products that are rapidly excreted. Rats excrete about 75% of an orally administered dose within the first 24 hours, primarily in urine (70%), with lesser amounts (5%) in feces. In mice, about one-third of a dermal dose was excreted in urine, with two-thirds in feces.

IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as carbofuran from a variety of sources, including drinking water, food, ambient air, occupational settings, and consumer products. This analysis of human exposure to carbofuran is limited to drinking water, food, and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works, and travels, what one eats, and physiologic characteristics related to age, sex, and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Detailed information concerning the occurrence of and exposure to carbofuran in the environment is presented in another document entitled "Occurrence of Pesticides in Drinking Water, Food, and Air" (Johnston et al. 1984). This chapter summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food, and air.

In the Exposure Estimation section of this chapter, available information is presented on the range of human exposure and intake for carbofuran from drinking water, food, and ambient air for the 70-kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combined exposures from those three sources.

Exposure Estimation

a. Drinking Water

No data were obtained on levels of carbofuran in drinking water. Therefore, the intake of carbofuran from drinking water could not be estimated.

b. Diet

Data obtained on levels of carbofuran in foods in the United States were insufficient for use in estimating typical dietary intake levels for carbo-

furan. However, a worst-case estimate of dietary intake [Theoretical Maximum Residue Contribution (TMRC)] was calculated based on tolerances set by EPA for carbofuran and its metabolites in and on raw agricultural commodities (Table IV-I) (FDA 1981). The TMRC was derived by multiplying the tolerance level for each specific commodity by an EPA food factor (based on the percent of the diet each food represents) and by a factor of 1.5 kg for daily intake, and then summing across commodities. Using all the commodities for which tolerances have been established (53% of the diet), the estimated TMRC was 232 ug/day (or 3.3 ug/kg/day for a 70-kg adult male). Using tolerance levels for only those foods posing the greatest theoretical residue contributions (potatoes, corn, rice, milk and dairy, bananas, sugar, and meat) (51% of the diet), the intake value calculated was similar (226 ug/day, or 3.2 ug/kg/day for a 70-kg adult male). Although the TMRC is near the EPA acceptable daily intake (ADI) of 5.0 ug/kg/day (FDA 1981), it would not be expected that individuals would be exposed to all the foods listed in Table IV-I at their tolerance levels.

c. Air

No data were obtained on levels of carbofuran in ambient air. Therefore, the intake of carbofuran from ambient air could not be estimated.

Summary

Data on the intake of carbofuran from drinking water, food, and ambient air are insufficient for use in determining which of the three sources is the major contributor to total intake.

Table IV-I. Tolerances for Carbofuran In and On Raw Agricultural Commodities

Commodity	Tolerance (ug/kg)	
	Carbamates[a]	Carbamates and metabolites[b]
Alfalfa		
fresh	5,000	10,000
hay	20,000	40,000
Bananas	100	100
Beets		
sugar	100	100
sugar, tops	1,000	2,000
Cattle		
fat	20	50
meat by-products	20	50
meat	20	50
Coffee beans	100	100
Corn		
fodder	5,000	25,000
forage	5,000	25,000
grain	100	200
Goats		
fat	20	50
meat by-products	20	50
meat	20	50
Hogs		
fat	20	50
meat by-products	20	50
meat	20	50
Horses		
fat	20	50
meat by-products	20	50
meat	20	50
Milk	20	100
Peanuts	100	200
hulls	1,000	5,000
Peppers	200	1,000

Table IV-I. Tolerances for Carbofuran In and On Raw Agricultural Commodities (Continued)

Commodity	Tolerance (ug/kg)	
	Carbamates[a]	Carbamates and metabolites[b]
Potatoes	1,000	2,000
Rice	200	200
straw	200	1,000
Sheep		
fat	20	50
meat by-products	20	50
meat	20	50
Sorghum		
fodder	500	3,000
grain	100	100
forage	500	3,000
Strawberries	200	500
Sugarcane	100	100

[a]Carbofuran and its carbamate metabolite.

[b]Carbofuran and its carbamate and phenolic metabolites.

Source: 40 CFR 180.254, July 1, 1981

REFERENCES

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Johnston P, Letkiewicz F, Borum D, Gambal N, Gerner G, et al. 1984. Occurrence of pesticides in drinking water, food, and air. Interim draft report. Prepared by JRB Associates, McLean, VA, for Office of Drinking Water, U.S. Environmental Protection Agency, Washington, DC.

V. HEALTH EFFECTS IN ANIMALS

A. Acute Toxicity

The acute toxic effects, especially lethality, resulting from exposure to carbofuran are largely attributable to inhibition of the enzyme acetylcholinesterase. This enzyme is an essential component in the synaptic transmission of nerve impulses in all animals, including humans and insects. Unlike some acetylcholinesterase inhibitors, carbofuran acts directly and does not require metabolic alteration. Some organophosphates, for example, have a latent period of several hours between absorption and appearance of signs of toxicity, whereas carbofuran, being a direct inhibitor, produces signs and symptoms within 30 minutes after absorption (Tobin 1970).

1. Lethality

The data from the published and unpublished studies of the lethal effects of single oral and dermal doses of carbofuran are listed in Table V-1. Numerous unpublished studies on the acute toxicity of carbofuran conducted by the FMC Corporation are cited in the review by the Midwest Research Institute (1976). The oral LD_{50} ranged from 6.4 to 14.1 mg/kg in rats, and from 25.0 to 38.9 mg/kg for the white Leghorn chicken.

Table V-1 Acute Oral Toxicity of Carbofuran

Species	Effect	Dose (mg/kg)	Reference
Rat	8/32[a]	2.5	Cambon et al. 1979
Rat[b]	LD_{50}	6.4 - 14.1	Midwest Research Institute 1976
Mouse	LD_{50}	2.0	Fahmy et al. 1970
Dog[b]	2/10[a]	18.85	Midwest Research Institute 1976
Chick (10 to 12 days old)	LD_{50}	6.3	Sherman et al. 1967
Chicken[b]	LD_{50}	25.0 - 38.9	Midwest Research Institute 1976
Mallard (adult)	LD_{50}	0.415 0.7 (male) 0.86 (female)	Hudson et al. 1972 FMC Corp. 1972
Fulvous Whistling Ducks	LD_{50}	0.238	Tucker and Crabtree 1970
Bobwhite quail	LD_{50}	8.0	FMC Corp. 1972
Japanese quail	LD_{50}	4.9 (male) 3.5 (female)	FMC Corp. 1972
Pheasant	LD_{50}	4.0 (male) 6.2 (female)	FMC Corp. 1972
Quelea	LD_{50}	0.42	Schafer et al. 1973
House Sparrow	LD_{50}	1.3	Schafer et al. 1973
Red-winged Blackbird	LD_{50}	0.42	Schafer et al. 1973

[a]Number dead/number exposed.
[b]Numbers reported without documentation from unpublished studies.

The beagle dog appeared more resistant than the rat. Chickens were more resistant than other bird species. The data on wildlife are presented because of the paucity of published, peer-reviewed studies on laboratory animals.

Wolfe and Escher (1980) determined lethal levels of carbofuran in mice at dietary levels of 250 and 500 mg/kg diet, or approximately daily doses of 49 or 98 mg/kg bw, calculated using a reported body weight of 13.8 g and a daily food consumption of 2.7 g/day. After four days, mortality was 20% at the 250-mg/kg (49 mg/kg/day) dose level and 100% at the 500-mg/kg (98 mg/kg/day) level.

Tobin (1970) reported a dermal LD_{50} value in rabbits of 10,200 mg/kg for technical grade carbofuran and >10,000 mg/kg for 10% granular carbofuran. The acute dermal LD_{50} in rabbits was much lower (14.7 mg/kg) when carbofuran was applied in organic solvent (Dowanol DMP) (Midwest Research Institute 1976). A summary of acute inhalation lethality data is given in Table V-2.

2. General Effects

The toxic effects of carbofuran are a result of its inhibition of the enzyme acetylcholinesterase. Mount and Oehme (1981) studied the effect of carbofuran and other pesticides on brain cholinesterase activity in cattle, swine and sheep. Brains of cattle (one year to adult) were removed at slaughter and homogenized for cholinesterase assay. A number of the animals

Table V-2 Inhalation Acute Toxicity Studies of Carbofuran[a]

Test animal	Sex	Formulation	Dust or aerosal	LD_{50}[b]
Rat	Mixed	80 WP	Dust	85
	Mixed	50 WP	Dust	120
	Male	50 WP	Dust	108
	Female	50 WP	Dust	133
Guinea Pig	Mixed	50 WP	Dust	74
	Male	50 WP	Dust	74
	Female	50 WP	Dust	53
	Mixed	75 WP	Aerosal	53
	Male	75 WP	Aerosal	43
	Female	75 WP	Aerosal	65
	Mixed	75 WP	Dust	43
	Male	75 WP	Dust	53
	Female	75 WP	Dust	45
Dog	Male	50 WP	Dust	52

[a]Experimental details are not provided for these otherwise unpublished studies.
[b]In mg/m^3 air.

Adapted from Tobin (1970).

were known to be "poisoned" with carbofuran (dose unspecified). Brain cholinesterase activity from these poisoned animals was depressed 71% compared to control animals.

Despite the fact that the mechanism of action of carbofuran is acetylcholinesterase inhibition within the nervous system and at the neuromuscular junction, most studies use cholinesterase activity in the blood as an indicator. Cambon et al. (1979) administered carbofuran to groups of pregnant Sprague-Dawley rats on gestation day 18. Eight animals per group were administered either 0.05, 0.25 or 2.5 mg/kg carbofuran by gastric intubation. The rats were killed 1, 5 or 24 hours after dosing and the activity of acetylcholinesterase was measured in both fetal and maternal blood, brain and liver. Toxic signs appeared at the highest dose (2.5 mg/kg) within five minutes. Mortality was high in this group. The results after one hour are summarized in Table V-3. The activity of acetylcholinesterase was significantly ($P<0.05$) reduced in all maternal and fetal tissue samples taken from surviving dams one hour after treatment with 2.5 mg/kg carbofuran. For lower doses, inhibition was found only in certain tissues one hour after treatment. There was a semiquantitative correlation between acetylcholinesterase inhibition in blood and in brain (nervous system). Differential inhibition of isoenzymes of acetylcholinesterase was observed in both maternal and fetal brain (Cambon et al. 1980).

A number of researchers have explored the potential of carbofuran to affect various biochemical parameters other than acetylcholinesterase.

Table V-3 Activity of Acetylcholinesterase in Maternal and Fetal Tissues from Rats Administered Carbofuran by Gastric Intubation

Dose (mg/kg)	Time	Acetylcholinesterase units per gram protein		Acetylcholinesterase units per gram of organ			
		Maternal blood	Fetal blood	Maternal brain	Maternal liver	Fetal brain	Fetal liver
Control	18 days	22.9±1.47	24.0±2.42	9.41±1.45	5.89±0.86	1.45±0.06	1.75±0.07
	19 days	23.7±1.06	17.7±1.48	9.30±1.70	3.70±1.18	1.39±0.10	1.35±0.11
0.05	1 hour	19.6±2.33[a]	16.4±3.70[b]	7.93±1.68	4.08±0.87[b]	1.42±0.11	1.81±0.22
0.25	1 hour	19.3±2.62[a]	17.5±3.15[b]	6.46±1.28[a]	5.43±1.06	1.45±0.06	1.44±0.22[a]
	5 hours	22.2±2.17	19.7±4.78[c]	8.16±2.00	4.60±0.95[c]	1.57±0.17	1.37±0.20[b]
	24 hours	23.6±3.49	18.7±3.97	7.42±1.25	4.84±0.40	1.55±0.02	1.38±0.09
2.50	30 min		14.7±2.84[b]	2.54±0.48[b]		1.17±0.11[b]	1.07±0.13[b]
	1 hour	18.3±2.18[b]	14.9±1.38[b]	4.11±1.03[b]	4.66±0.97[c]	1.24±0.15[c]	1.08±0.36[b]
	5 hours	21.0±1.53[c]	19.2±5.11[c]	6.49±2.40[c]	4.06±0.75[b]	1.27±0.30	1.37±0.25[a]
	24 hours	24.3±2.85	15.8±2.39	8.44±2.03	4.56±1.13	1.77±0.36	1.47±0.16

[a]Significantly different from control at P<0.01.
[b]Significantly different from control at P<0.001.
[c]Significantly different from control at P<0.05.

Adapted from Cambon et al. (1979).

Cranmer et al. (1978) measured several endocrine system parameters in mice exposed *in utero* to two levels of carbofuran. Adult female F_2 hybrid mice were fed daily doses of 0.01 or 0.50 mg/kg bw carbofuran (technical grade) in peanut butter. Doses chosen were 0.02% and 1% of the minimum dose which caused increased terata or morbidity in pups as determined in a pilot study. Treatment began the day after mating and continued throughout the entire gestation period (22 days). Dams of all groups gave birth to approximately equal numbers of normal offspring. Exposure of pups through mother's milk may have occurred, since this was not controlled. At 101 days of age, pups exposed *in utero* were sacrificed for endocrinological evaluation. Levels of plasma corticosterone were measured in resting mice and mice stressed by swimming in 2°C water. *In vitro* corticosterone production by excised adrenals was measured for resting mice. Liver capacity to metabolize exogenous corticosterone was measured three ways: as side-chain reduction, as A-ring reduction or as the two together. Two independent control groups were examined concurrently. Group size was 8 to 11 except for the stressed groups, which had 3 to 5 mice. Differences in group means were detected by either Student's t-test or Duncan's multiple range test. Significant differences had P values below 0.05.

No differences were observed for any of the high-dose mice, male or female. The low-dose females showed increased resting plasma corticosterone levels and increased liver weight (which was proportional to the observed body weight increase). Low-dose females also exhibited decreased total liver metabolic activity, but neither individual metabolic route differed

significantly from the controls. The low-dose males exhibited decreased liver metabolic activity by all three measures. While the reasons for lack of a dose-response relationship in this experiment are unclear, the authors maintain that such complex dose-response curves reflect a real biological phenomenon. There are a number of examples in the literature of such dose-response inversions following prenatal exposure to toxic compounds (Cranmer et al. 1978).

Several studies have investigated the interaction of carbofuran with the immune system. Street and Sharma (1975) administered carbofuran in feed to groups of seven male New Zealand rabbits (2 kg). Doses of 0, 0.03, 0.16, 0.49 or 1.05 mg/kg/day of carbofuran (equivalent to 0, 0.5, 2.5, 9.0 or 20.0 mg/kg in feed) were administered for eight weeks. During the final four weeks, animals were tested for immunological response to an antigenic challenge and checked for gross abnormalities. Baseline blood counts and body weights were determined during the first 33 days. At day 33, animals were given subcutaneous injections of sheep erythrocytes in Freund's complete adjuvant. Complement-dependent hemolysin titers, hemagglutination titers and immunoglobulin levels (expressed as immunoglobulin/transferrin ratios) were determined 10, 17 and 24 days after immunization, as well as before immunization. No significant changes were observed in either titers or immunoglobulin/transferrin ratios after immunization for any dose, nor were any significant changes observed for total or differential leukocyte counts at any time.

Tuberculin sensitivity was tested by a skin test 10 and 24 days after immunization. Sensitivity (expressed as weal size compared to control group) was significantly decreased for all doses at day 10, but by day 24, only one group (0.49 mg/kg/day) remained significantly decreased.

Animals were sacrificed at day 57, and ratios of organ weight to body weight were calculated for liver, kidney, spleen and adrenal gland. Only the highest dose group (1.05 mg/kg/day) exhibited any significant changes (slight enlargement of the adrenal gland and spleen). No significant body weight changes were observed at any point for any group. No lesions were found in sections of liver, kidney and adrenal. Lymph node sections were also examined for the ability to bind fluoresceinated goat anti-rabbit globulin: the two highest doses showed decreased binding. Significant decreases in germinal centers in stained sections of spleen were found for all dose groups except the 0.49 mg/kg/day group. No significant atrophy was observed in stained thymus sections.

Several changes were observed by the authors to be "large, but not statistically significant." A transient suppression of cell-mediated immunity was observed for carbofuran in the decreased size of the skin weal in a tuberculin test. This by itself is not a strong indication of immunosuppression, and the data presented cannot be interpreted as indicating that carbofuran is significantly immunosuppressive.

Barnett et al. (1980) studied the effect of carbofuran on development and serum immunoglobulin levels in mice. Pregnant female mice (F_2 dihybrid) were administered daily doses of carbofuran at 0, 0.01 or 0.50 mg/kg bw (43, 23 and 18 animals per group, respectively in the diet throughout gestation). The doses were 0.02% and 1%, respectively, of the minimum dose causing increased terata or morbidity in pups as determined in a pilot study. Dams in all groups gave birth to approximately equal numbers of offspring. Offspring treatment groups were then monitored for differences in viability and body weight from birth to 800 days (311, 145 and 134 pups for each respective dose group). Serum immunoglobulin levels were measured by radial immunodiffusion for animals sacrificed at 101, 400 and 800 days.

There was no observed effect on viability, average lifespan or incidence of any single cause of death for either carbofuran dose. A transient effect on body weight was observed for pups born to mothers exposed to 0.5 mg/kg/day, (pup mean weight was lower than the control group at birth, 7 and 14 days). No body weight differences were observed at 28 days or for the remainder of the lifespan. No body weight differences were observed for the lower dose animals.

Measurement of serum IgG_1, IgG_{2a}, IgG_{2b}, IgM and IgA revealed dose-related effects of carbofuran on immunoglobulin levels in one case. Male pups prenatally exposed to the higher dose (0.5 mg/kg/day) exhibited elevation of IgG_1 at 101 and 400 days, but not at 800 days. A transient, non-dose-related effect was seen for females; decreased serum IgG_1 and IgG_{2a} levels were

observed at 101 days, but only in low-dose animals. Additionally, two inconsistent increases in IgA levels in females were observed at 101 days for the high-dose group and at 400 days for the low-dose group. No other significant differences were observed for any other values compared, including IgM and IgG_{2b}. This study has led to the listing of carbofuran as a developmental immunotoxicant (Roberts and Chapman 1981).

Several hematological parameters were evaluated in mice treated with carbofuran on a subacute basis (Gupta et al. 1982). A group of 15 male Swiss mice (four to six weeks old, 20 to 25 g) were given weekly injections of 0.25 mg/kg carbofuran for six weeks. Carbofuran was dissolved in normal saline and injected intraperitoneally. A control group of 15 mice received the same volume of saline. Mice were sacrificed and parameters were measured 18 hours after the sixth injection. Most of the parameters measured were significantly affected by carbofuran treatment (Table V-4).

The observed changes are consistent with three gross effects, as suggested by the authors. They included liver damage (evidenced by the prolongation of clotting time), bone marrow damage (leading to macrocytic anemia) and possible compensation by the spleen for bone marrow damage.

B. Chronic Toxicity

Chronic administration of carbofuran did not result in any cumulative toxic effects. Rotaru at al. (1981) fed male Wistar rats (initial weight

Table V-4 Hematological Parameters of Mice Administered Carbofuran or Normal Saline

Parameter	Normal saline	Carbofuran	% Change
Clotting time	29.5	116[a]	+293
Hemoglobin content in g/100 mL whole blood	13.14	9.1[a]	-31
Total count of RBC/mm^3	10.7×10^6	2.96×10^{6a}	-72
Total count of WBC/mm^3	4,863	7,063[a]	+45
Platelet count/mm^3	$1,348 \times 10^3$	433×10^{3a}	-68
Erythrocyte sedimentation rate (mm/h)	1.6	1.06[a]	-34
Hematocrit value (%)	42.71	17.49[a]	-59
Mean corpuscular volume (μm^3)	39.91	58.3[a]	+46
Mean corpuscular hemoglobin (pg)	12.28	34.88[a]	+184
Mean corpuscular hemoglobin concentration (%)	30.76	47.23[a]	+54
Lymphocytes			
a. Small	55.4	42.4[a]	-23
b. Large	16.8	23.5[a]	+40
Monocytes	5.7	4.9	-14
Basophils	0.9	2.7[a]	+200
Neutrophils	20.3	26.3[a]	+30
Total count of bone marrow cells/femur	8.4×10^6	6.02×10^{6a}	-28

continued-

[a]Value significantly different from control, $P<0.01$; Student's t-test.

Table V-4 - continued

Parameter	Normal saline	Carbofuran	% Change
Erythrocyte precursors of bone marrow/femur	1.2×10^6	0.36×10^{6a}	-70
WBC precursors of bone marrow/femur	6.5×10^6	5.63×10^{6a}	-13
Megakaryocyte precursors of bone marrow/femur	0.7×10^6	ND[b]	--
Weight of spleen (mg)	118.8	113.2	-5
Total count of splenic cells/spleen	2.1×10^8	7.8×10^{8a}	+271

[a]Value significantly different from control, $P<0.01$, Student's t-test.
[b]ND = None detected.

Adapted from Gupta et al. (1982).

75 g) carbofuran in the diet for 180 days at levels of 0, 10 or 25 mg/kg (ten animals per group). These levels are approximately 0, 0.66 and 1.58 mg/kg/day, calculated using reported final body weights of 213 g and 221 g, respectively, and assuming a daily food consumption of 0.014 kg (Arrington 1972). The animals were examined histopathologically after sacrifice, and the activity of hepatic enzymes was measured.

Serum enzymes examined included lactic dehydrogenase (LDH), glutamate dehydrogenase, gamma-glutamyl transpeptidase, glutamate oxalate transaminase, glutamate pyruvate transaminase, and alkaline phosphatase. No differences in weight increase or liver weights were observed between controls and treated animals during the experiment. The minor changes observed in liver morphology seemed to relate more to increased caloric intake associated with feeding ad libitum than to the level of carbofuran administered. The only serum enzyme showing a significant ($P<0.01$) increase was LDH; the increase was observed only at 10 mg/kg (0.66 mg/kg/day) carbofuran. Thus, there appeared to be no dose-response relationship for serum enzymes and carbofuran.

Wolfe and Esher (1980) fed old-field mice (Peromyscus polionotus) and cotton mice (P. gossypinus) 100 mg carbofuran/kg for eight months in order to assess toxic effects. This dose level corresponds to approximately 19.6 mg/kg/day for P. polionotus and 12.2 mg/kg/day for P. gossypinus (based on reported body weights of 13.8 g or 29.5 g and reported daily food consumptions of 2.7 or 3.6 g, for each respective strain). Food consumption, mortality, reproduction and development of young were monitored throughout the

duration of the experiment. Male survivors were tested for activity in a behavioral maze. There were no indications of cumulative or delayed effects, except for delayed mortality in P. polionotus.

In a one-year dietary study (FMC Corporation 1983), four groups of beagle dogs were administered daily doses of 0, 10, 20 or 500 mg/kg carbofuran in the diet, or approximately 0, 0.25, 0.50 or 12.5 mg/kg/day, assuming a body weight of 16 kg and daily food consumption of 0.4 kg (Arrington 1972). The animals were allowed access to the treated food for two hours each day. Each group consisted of six males and six females. Of the various biochemical, hematological and clinical observations made, the only positive result was depression of plasma cholinesterase at the higher dose levels. No biologically significant effects were obsetved at the 10-mg/kg (0.25 mg/kg/day) level. At 20 mg/kg (0.50 mg/kg/day), there was a consistent, significant depression of plasma cholinesterase in males; therefore, this level is the LOAEL. At 500 mg/kg (12.5 mg/kg/day), there was marked depression of plasma cholinesterase in both males and females and a consistent reduction in erythrocyte cholinesterase.

C. Reproductive/Teratogenic Effects

In a one-year dietary study (FMC Corporation 1983), four groups of beagle dogs were administered daily doses of 0, 10, 20 or 500 mg/kg carbofuran in the diet, or approximately 0, 0.25, 0.50 or 12.5 mg/kg/day, assuming a body weight of 16 kg and daily food consumption of 0.4 kg (Arrington 1972). The animals

were allowed access to the treated food for two hours each day. Each group consisted of six males and six females. There was a treatment-related testicular degeneration with some aspermia in four of the five males at the 500 mg/kg (12.5 mg/kg/day) dose and in one of six males at the 20-mg/kg (0.50 mg/kg/day) dose. In females at the high-dose level, uterine hyperplasia and hydrometria were observed.

In another study (FMC Corporation 1980b), rats were treated with dietary carbofuran for three generations to ascertain reproductive effects. Weanling Charles River rats were fed 0, 20 or 100 mg/kg dietary carbofuran, corresponding to approximately 0, 1.0 or 5.0 mg/kg/day. Generations one and two consisted of 10 males and 20 females per group, and generation three contained 24 females. No effect on either female or male fertility or on the length of gestation was observed at either dose level. Litter size and growth were comparable to controls in both treatment groups in all three generations. The viability and survival of pups were largely unaffected by carbofuran, except that survival of the first litter in all three generations in the 100-mg/kg group (5.0 mg/kg/day) was slightly lower by day four of lactation. Therefore, in this study it was concluded that the NOAEL for carbofuran, using reproductive effects as an endpoint, is 20 mg/kg, corresponding to 1.0 mg/kg/day carbofuran.

In studies on the immunocompetence of mice exposed to carbofuran *in utero*, data on reproductive effects of the compound were obtained (Barnett et al. 1980). These results are presented in Table V-5. No significant ($P>0.05$)

Table V-5 Mortality of Offspring from Mice Receiving Carbofuran During Gestation

Dose (mg/kg/day)	Number of dams	Number of pups born	% Mortality to 28 days	% Mortality to 800 days
0	43	311	6	33
0.01	23	145	5	29
0.50	18	134	6	27

Adapted from Barnett et al. (1980).

effects on the number of pups born or on mortality of pups at 28 or 800 days were observed when the dams were administered either 0.01 or 0.50 mg/kg/day throughout gestation.

The FMC Corporation conducted a complete series of teratology studies with carbofuran in the rat (dietary exposure) and in the rabbit (oral gavage). In a preliminary experiment (FMC Corporation 1980a), pregnant female Charles River CD rats were fed diets containing 0, 20, 60, 120, 160 or 200 mg/kg of carbofuran during gestation days six through nineteen. These dose levels correspond to approximately 0, 1.0, 2.9, 5.8, 7.7 or 9.7 mg/kg/day, assuming a body weight of 0.290 kg and a food consumption of 0.014 kg/day (Arrington 1972). There was 100% survival in all groups. No clinical symptoms or uterine abnormalities were observed during the first two days of treatment in the 60, 120, 160 and 200 mg/kg groups. Maternal body weight gain was decreased in the 120, 160 and 200 mg/kg groups. On the basis of this study, a complete teratology study was conducted (FMC Corporation 1981a). Forty pregnant female Charles River CD rats were fed carbofuran at 0, 20, 60 or 160 mg/kg diet (daily doses of 0, 1, 3 or 8 mg/kg bw) during gestation days six through nineteen. One-half of the fetuses were examined for visceral and skeletal abnormalities on gestation day 20. Remaining dams delivered normally and the pups were mothered until lactation day 21. There were no clinical signs of carbofuran toxicity in any group. Food consumption was normal in the 20 and 60 mg/kg (1 and 3 mg/kg/day) group and was reduced 6% in the 160 mg/kg (8 mg/kg/day) group. Maternal body weight gains were significantly reduced in 60 and 160 mg/kg (3 and 8 mg/kg/day) groups during the treatment period; no

such effect was seen in the 20 mg/kg (1 mg/kg/day) group. During the lactation period, mean body weight gain in all treatment groups was normal. There were no effects on length of gestation, parturition, the physical appearance of pups or any pup survival indices. The mean pup body weights in the 60 and 160 mg/kg (3 and 8 mg/kg/day) groups were slightly reduced, but this was attributed to the reduction in maternal body weights in those groups. No terata were observed in any group. On the basis of this study, 20 mg/kg (1.0 mg/kg/day) was determined to be the NOAEL in rats, with respect to the effects observed.

In the rabbit study (FMC Corporation 1981b), seven-month-old female New Zealand rabbits were treated (by oral gavage) at 0, 0.12, 0.5 or 2.0 mg/kg/day on days six through eighteen of gestation. One of the dams in the high-dose group died on the eleventh day of gestation from unknown causes. The other animals in that group experienced a 20% reduction in body weight gain during the treatment period. No terata were observed in any of the treatment groups, and there were no differences in the number of fetuses or litters with developmental or genetic abnormalities in any of the carbofuran-treated groups when compared to the control group. Three dams aborted toward the end of the gestation period, one from each of the treatment groups. No significance with respect to carbofuran treatment was attributed to this fact. Thus, this study indicated no teratogenic effects of carbofuran in rabbits at the dose levels used.

D. Mutagenicity

There have been many studies of the mutagenicity of carbofuran using short-term tests. These are summarized in Table V-6.

Waters et al. (1982) employed seven genotoxicity assays in testing technical-grade carbofuran for mutagenicity. These assays included both prokaryotic and eukaryotic systems and were capable of detecting point mutations and primary DNA damage. Carbofuran gave negative results in five of the seven assays and inconclusive results in the remaining two.

Two bacterial reversion assays were used. Five strains of Salmonella typhimurium, TA98, TA100, TA1535, TA1537 and TA1538, were used in a plate incorporation assay. Escherichia coli strain WP2 was used in a similar reversion assay. At least six doses, up to 10 mg/plate, were tested for each strain, with and without S9 activating system (Aroclor-1254-induced rat). No dose-dependent increase in reversion rate was observed in either assay.

Three eukaryotic assays were used. A sex-linked recessive lethal assay employing Drosophila melanogaster was utilized. Carbofuran elicited less than 0.2% increase in the spontaneous mutation rate. A mitotic recombination assay in the yeast Saccharomyces cerevisiae (strain D7) was also used. No activity was seen for carbofuran at any of the five doses, with or without S9 activation. A variation of Simmon's unscheduled DNA synthesis assay was used employing WI-38 human lung fibroblast cells. None of the five

Table V-6 Short-Term Assays for Mutagenicity of Carbofuran

Test	Dose[a]	Results	Reference
Ames (strains)			
TA98,100,1535,1537,1538	up to 10 mg/plate (T)	-,-[b]	Waters et al. 1982
TA98,100,1535,1537,1538	10 µg/plate (?) (T)	-,-[b]	Gentile et al. 1982
TA98,100,1535,1537,1538	10 µg/plate (C)	-,-[b,c]	Gentile et al. 1982
TA98,1538	up to 10 mg/plate	+,+[b]	Moriya et al. 1983
TA100,1535,1537	up to 5 mg/plate	-	Moriya et al. 1983
TA98	NT[d]	+	Shirasu et al. 1982
TA98,100,1537,1538	NT	-,-[b]	Poole et al. 1977
TA98,100	up to 25 µg/plate (A)	-,-[b]	Nelson et al. 1981
Other Tests			
E. coli WP2 reversion	up to 10 mg/plate (T)	-,-[b]	Waters et al. 1982
E. coli WP2 reversion	up to 5 mg/plate (T)	-	Moriya et al. 1983
E. coli WP2 reversion	NT	-,-[b]	Poole et al. 1977
E. coli reversion	NT	-	Simmons et al. 1979
E. coli, B. subtilis differential toxicity	NT	-	Simmons et al. 1979
Yeast mitotic recombination	NT (T)	-	Waters et al. 1982
Yeast gene conversion	over 2-3 logs (T,C)	-,-[b,c]	Gentile et al. 1982

continued-

[a]Grade of carbofuran: (T) = technical, (C) = commercial, (A) = 99.5% analytical.
[b,c]With metabolic activation by rat S9 (b) or plant homogenate (c).
[d]NT = No test (mixed or ambiguous results).

Table V-6 - continued

Test	Dose[a]	Results	Reference
Yeast mitotic recombination	NT	-	Poole et al. 1977
Z. mays back mutation	2.24 kg/ha (C)	-	Gentile et al. 1982
D. melanogaster recessive lethal	NT (T)	-	Waters et al. 1982
CHO V79 cells	NT	+,-[b]	Wojciechowski et al. 1982
UDS Human lung fibroblasts	5 doses (T)	-,-[b]	Waters et al. 1982
UDS Human fibroblast	NT	-	Simmons et al. 1979

[a]Grade of carbofuran: (T) = technical, (C) = commercial, (A) = 99.5% analytical.
[b,c]With metabolic activation by rat S9 (b) or plant homogenate (c).
[d]NT = No test (mixed or ambiguous results).

carbofuran concentrations tested caused a significant increase in ^{3}H-labeled thymidine incorporation into synchronized WI-38 cells, with or without S9.

Two bacterial assays for primary DNA damage also were performed. Both assays depended on the differential toxicity between two bacterial strains, one of which is deficient in the ability to repair DNA. One assay used E. coli strains p3478 and W3110, while the other used Bacillus subtilis strains M45 and H17. Results of both of these assays were inconclusive.

Moriya et al. (1983) tested carbofuran (of unspecified purity) in two bacterial reversion mutagenesis assay systems according to the Ames method. A positive dose-response relationship for S. typhimurium strains TA1538 and TA98 was reported, with no effect caused by the addition of S9 mix. Doses tested for TA1538 were implied to be the same as for TA98. The reversion rate for TA98 was calculated to be 0.0023 revertants per nmole. No increase in reversion rate was observed for carbofuran tested on S. typhimurium strains TA100, TA1535 and TA1537 and on E. coli strain WP2 hcr up to a dose of 5 mg/plate. Testing on the above four strains was implied to have been done with and without S9 mix.

Gentile et al. (1982) conducted mutagenicity tests for ten insecticides using S. typhimurium and S. cerevisiae following plant or animal activation. The plant activating system was the soluble fraction (10 minutes, 1,000 x g supernatant) of homogenized Zea mays plants. Carbofuran was negative for genotoxic properties in all tests.

Carbofuran was tested to ascertain its effect on chlorophyll a synthesis and nitrogen fixation in five cyanobacterial forms belonging to the genera Hapalosiphon, Westiellopsis and Calothrix (Kaushik and Venkataraman 1983). No effects were observed in cultures at final concentrations of 1 and 10 mg/L, while minimal inhibition of chlorophyll a was observed in most species at 100 mg/L.

N-Nitrosocarbofuran has also been extensively tested for its mutagenic potential in several short-term assays. These results are summarized in Table V-7.

Nelson et al. (1981) obtained 99.5% pure carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran and prepared N-nitroso derivatives of each. Mutagenicity for all six chemicals was tested using S. typhimurium strains TA98 and TA100, with rat liver S9 fraction activation. The non-nitrosated carbofuran, 3-hydroxycarbofuran and 3-ketocarbofuran all failed to show mutagenic activity in either the TA98 or TA100 strain, with or without S9 activation, in parallel tests. However, all three of the nitroso derivatives were highly mutagenic without S9 activation in the TA100, but not in the TA98 strain. Addition of S9 fraction decreased mutagenic activity. Nelson et al. (1981) rank the nitroso derivatives of carbofuran and its metabolites among the most mutagenic compounds tested in the TA100 system to date.

Table V-7 Short-Term Assays for Mutagenicity of N-Nitrosocarbofuran

Test	Dose	Results	Reference
Ames (strains)			
TA1535	1.0 to 1,000 µg/plate	+	Lijinsky & Andrews 1979
TA100,1535	0.1 to 100 µg/plate	+,-[a]	Rickard et al. 1982
TA98	0.1 to 100 µg/plate	-,-[a]	Rickard et al. 1982
TA98	2 to 120 ng/plate	-	Nelson et al. 1980
TA100	2 to 120 ng/plate	+	Nelson et al. 1980
TA100	up to 25 µg/plate	+	Nelson et al. 1981
Other Tests			
SCE - CHO cells	10^{-9} to 5 x 10^{-5}M	+	Nelson et al. 1981
CHO cells chromosome aberrations	5 x 10^{-8} to 10^{-5}M	+	Nelson et al. 1981

[a]With rat S9 metabolic activation.

E. Carcinogenicity

The FMC Corporation tested carbofuran for carcinogenicity in both rats and mice. In neither case was there a significant increase in tumors of any kind attributed to carbofuran when compared with controls.

In the rat study (FMC Corporation 1980d), Charles River CD rats were administered carbofuran at 0, 10, 20 or 100 mg/kg in the diet for two years, corresponding to approximately 0, 0.5, 1.0 or 5 mg/kg/day. Ninety animals per sex per dose were utilized in the study. Parameters observed included body weight, food consumption, behavior, ophthalmoscopy, hematology, biochemistry, urinalysis and histopathology. The only statistically significant change observed was slightly decreased group mean body weights for males at 100 mg/kg throughout the study. The 10- and 20-mg/kg groups and all females were comparable to controls throughout the study. There were no effects on cholinesterase at 10 or 20 mg/kg, while at 100 mg/kg there was some statistically and biologically significant plasma, red blood cell and brain cholinesterase depression. In this study, those neoplasms and non-neoplastic histologic changes which were observed were judged to be spontaneous lesions unrelated to the administration of or exposure to carbofuran during the two-year treatment period.

In the mouse study (FMC Corporation 1980c), Charles River CD-1 mice were fed carbofuran at 0, 20, 125 or 500 mg/kg in the diet, or approximately 0, 3, 18.8 or 75 mg/kg/day. One hundred animals per sex per dose were utilized

in this two-year study. Again, body weight, food consumption, behavior, hematology, biochemistry, urinalysis and histopathology were observed. The results indicated some effects on body weight at the highest dose (75 mg/kg bw). There was a significant decrease in body weight up to 78 weeks in females and up to 65 weeks in males. By the end of the experiment, however, there were no statistically significant differences in body weights between treated and control animals. Some intermittent reductions in food consumption occurred during the experimental period. At 18.8 and 75 mg/kg bw, statistically significant reductions in brain cholinesterase were observed in both males and females; there were no effects at lower doses. No significant effects on plasma or red blood cell cholinesterase were observed at any of dose levels during the experiment. Analysis of tumor incidence in the animals indicated no treatment-related tumorigenic influence or microscopic changes associated with the administration of carbofuran in organs in any of the carbofuran-treated mice.

Lijinsky and Schmahl (1978) administered N-nitroso derivatives of various agricultural chemicals, including nitrosocarbofuran, to six- to eight-week-old Sprague-Dawley rats. Sixteen male and 16 female rats were administered a solution of nitrosocarbofuran in corn oil at the rate of 66 mg/kg bw by gavage once a week. Treatments were carried out for ten consecutive weeks as a test for carcinogenicity. Three rats died due to liver toxicity after two treatments. The experiment was restarted with a new group of rats, at one-quarter the dose (16.5 mg/kg), once a week, for a total of 23 weeks, at which time six rats had died and treatments were terminated. Another group of

rats was given a lower dose, 10 mg/kg, by gastric intubation, weekly for ten weeks. A few rats were sacrificed when moribund. All other rats were allowed to survive until spontaneous death. Histological examinations for tumors were performed on each rat. The results are summarized in Table V-8. Stomach tumors were observed in 17 of the 26 rats given the 16.5-mg/kg dose. Three of the 12 rats given the 10 mg/kg dose developed stomach tumors. In contrast, none of the 40 control rats given weekly treatments with the oil vehicle alone developed any tumors.

F. Summary

The mode of action of carbofuran in animals is inhibition of acetylcholinesterase, and that enzyme is the most sensitive indicator of the acute effects of the compound. Symptoms of poisoning in animals reflect the inhibition of nerve synaptic acetylcholinesterase. Mammalian LD_{50} values for carbofuran range from 2.0 to 14.1 mg/kg. The toxicity to some birds is somewhat greater.

Other acute effects on the immune system and the blood have been reported. However, the effects reported are not severe, and are reversible when exposure to carbofuran is terminated.

Carbofuran was tested for carcinogenicity in both rats and mice, and no significant increases in tumors were observed.

Table V-8 Survival and Carcinomas in Rats Administered Nitrosocarbofuran by Gavage

No. of Weeks	Weekly dose (mg/kg)	Total dose (mg/kg)	Sex[a]	Number of rats surviving at week						Number of rats with stomach carcinomas	All Tumors
				0	20	40	60	80	100		
23	16.5	380	M	13	12	12	11	4	0	11	11
			F	13	8	8	6	2	0	6	7
10	10	100	F	12	8	4	4	3	1	3	3

[a]M = male; F = female.

Adapted from Lijinsky and Schmahl (1978).

Many short-term assays of mutagenic potential of carbofuran have been conducted. Most tests were negative, although there were a few positive results, particularly in S. typhimurium strains TA98 and TA1538. The N-nitroso derivative of carbofuran, which results from the chemical nitrosation of carbofuran by nitrite under acidic conditions, is both a potent mutagen and a cause of stomach cancer in rats. It must be emphasized that there is no evidence for the environmental or biological formation of this compound at the present time. Bioassays to determine the possible carcinogenicity of carbofuran in rats and mice have been negative.

There do not appear to be any teratological effects of carbofuran in laboratory animals, although there are some effects on the reproductive system. Aspermia and testicular degeneration in dogs and some minimal decreases in pup survival in rats have been observed. These effects were observed only at doses higher than the NOAEL based on cholinesterase inhibition.

VI. HEALTH EFFECTS IN HUMANS

Several cases of adverse effects in applicators and formulators using carbofuran have been reported (Tobin 1970). Exposed individuals experienced only mild symptoms of acetylcholinesterase depression, including a vague feeling of malaise, hyperhydrosis, light-headedness, nausea, blurring of vision, hypersalivation and vomiting (Tobin 1970). These symptoms were rapidly reversed when exposure ceased. Further symptoms which may occur with more severe poisoning include chest tightness, muscular twitching, convulsion and coma. Treatment with atropine appeared to be efficacious in the few reported cases where its use was necessary (Tobin 1970).

The N-methylcarbamates, of which carbofuran is an example, inhibit several aliesterases. However, there is no known toxic manifestation of this effect (Tobin 1970).

The only reports in the literature of exposure to carbofuran in humans under controlled conditions were described by FMC Corporation in its pesticide registration petition (FMC Corporation 1977). Human subjects were exposed to oral and dermal carbofuran doses in an experimental setting. A progression of oral doses beginning with 0.05 mg/kg was established for administration to healthy males (two subjects per dose). The compound was administered as a single dose following breakfast. At the lowest dose, 0.05 mg/kg, no symptoms were observed. At 0.10 mg/kg, symptoms included headache and a feeling of light-headedness which the investigators indicated may have been unrelated to the treatment. At 0.25 mg/kg, symptoms of acetylcholinesterase depression

were observed (dry mouth, salivation, diaphoresis, abdominal pain, drowsiness, dizziness, anxiety and vomiting). As a result, no higher doses were used.

In dermal studies, healthy males were treated after breakfast with carbofuran at doses beginning with 0.5 mg/kg/cm^2 in artificial sweat. The response of humans to carbofuran challenge appeared to be related to exercise, temperature and relative humidity. At 2 mg/kg under high temperature (85° to 95°F) and humidity (68% to 85%) with mild exercise, symptoms of cholinesterase depression were elicited. These symptoms correlated with 44% or more depression in erythrocyte cholinesterase. However, no symptoms were elicited by doses up to 32 mg/kg at low temperatures (70° to 75°F) and humidity (35% to 40%). Three days of sequential dosing at 1 mg/kg or less resulted in no cumulative cholinesterase depression.

An additional study has been conducted by FMC comparing cholinesterase inhibition and effects of Furadan 4F[a] and FMC 35001 4EC (Arnold 1978). In studies with healthy adult human males, median age 36, receiving a dermal application of carbofuran during a four-hour exposure period, the observed symptoms were those of cholinesterase inhibition (nausea, stomach cramps, vomiting, dizziness, weakness, dry lips, swollen tongue, increased salivation and tremors). Doses with the carbofuran formulation up to 4 mg/kg for four hours resulted in a 55% decrease in erythrocyte cholinesterase. There was no dose-response relationship for plasma cholinesterase depression in the dose range of 0.5, 1.0, 2.0 and 4.0 mg/kg.

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[a]Registered trademark.

VII. MECHANISM OF TOXICITY

A. Acetylcholinesterase Inhibition

As described earlier, carbofuran is a potent inhibitor of the enzyme acetylcholinesterase. It is a direct inhibitor (i.e., it does not require metabolic alteration to become active) (Tobin 1970).

Carbofuran, like the organophosphorous and other carbamate insecticides, acts on the nervous system by inhibiting acetylcholinesterase at the synapse (O'Brien 1976). Messages are transmitted across the nerve synapse by transmitter substances. Acetylcholine acts in that role in cholinergic junctions. When the impulse has passed, acetylcholinesterase acts on the acetylcholine to remove it from the system. The carbamates, including carbofuran, owe their activity to configurational similarity to acetylcholine and interaction with the active sites of acetylcholinesterase. Carbofuran serves as a substrate for the enzyme, carbamylating the catalytic site and thereby rendering the acetylcholinesterase inactive.

The structure of carbofuran includes a bulky side chain that is capable of interacting with one or more binding sites of cholinesterase located at an appropriate distance from the carbamyl group to accommodate the enzyme (O'Brien 1976). The carbamylated acetylcholinesterase is less stable than the

phosphorylated enzyme that results from reaction with organophosphate insecticides. Thus, its effect is more quickly reversible, and symptomatic recovery is more rapid.

B. Interactions

No studies in mammals of carbofuran interactions with other chemicals were found in the literature. Dieter and Ludke (1978) reported that morsodren (as methyl mercury) had a slight potentiating effect in quail. Methylene dioxyphenyl compounds interact synergistically with other carbamates, but no data on carbofuran were located.

C. Summary

The mechanism of action of carbofuran is that of the class of insecticides to which it belongs, the carbamates. Inhibition of acetylcholinesterase by carbofuran has been amply demonstrated, and it appears to behave as a conventional member of its group. There are no data on interactions of carbofuran with other compounds, although methylene dioxyphenyl compounds are potent synergists with other carbamates.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the non-carcinogenic and carcinogenic effects. In the quantification of non-carcinogenic effects, an Acceptable Daily Intake (ADI) is calculated. An Adjusted Acceptable Daily Intake (AADI) and Health Advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{(\text{NOAEL or LOAEL in mg/kg/day})(\text{Body Weight in kg})}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking Water Volume in L/day}} = \text{mg/L}$$

where:

NOAEL = no-observed-adverse-effect level.

LOAEL = lowest-observed-adverse-effect level.

Body weight = 70 kg for adult or 10 kg for child.

Drinking water volume = 2 L per day for adults or 1 L per day for children.

Uncertainty/Safety Factor = 10, 100 or 1,000.

Utilizing these equations, the following drinking water concentrations are developed for non-carcinogenic effects:

1. A one-day HA for 10-kg child.
2. A one-day HA for 70-kg adult.
3. A ten-day HA for 10-kg child.
4. A ten-day HA for 70-kg adult.
5. A lifetime AADI for a 70-kg adult.

The distinctions made between the HA calculations (items 1 through 4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1 to 2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon non-carcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS 1977, 1980) is typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.

- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.

- An uncertainty factor of 1,000 is used when limited or incomplete acute or chronic toxicity data are available or when only the acute or chronic toxicity data identifying a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines for selection of an uncertainty factor, but must be considered include: (1) the quality of the toxicology data, (2) the significance of the adverse effect and (3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen (there is insufficient evidence to classify carbofuran as a carcinogen following oral exposure), mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical via drinking water. The bioassay data used in these estimates

are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for non-continuous animal feeding, non-lifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 liters of water per day. The multi-stage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as (1) incomplete knowledge concerning the health effects of contaminants in drinking water, (2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study

results and (3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

A. Non-Carcinogenic Effects

Carbofuran, a typical carbamate insecticide, was absorbed rapidly after oral or dermal administration. Rats absorbed 63% of an oral dose from the stomach in 30 minutes and 72% of a dermal dose in 15 minutes (Ahdaya et al. 1981, Shah et al. 1981). Excretion was similarly rapid, with 75% of an oral dose being excreted within 24 hours after administration (Dorough 1968). Urinary excretion was the principal path of elimination.

The oral LD_{50} for carbofuran in mammals ranged from 2.0 to 14.1 mg/kg in rodents. Chickens were more tolerant; LD_{50} values ranged from 25 to 39 mg/kg. Other avian species were more sensitive; LD_{50} values ranged from 0.2 to 1.3 mg/kg (Sherman et al. 1967, Schafer et al. 1973, Midwest Research Institute 1976).

1. Short-Term Exposure

Cambon et al. (1979) administered carbofuran to groups of pregnant Sprague-Dawley rats on gestation day 18. Eight animals per group were administered either 0.05, 0.25 or 2.5 mg/kg carbofuran by gastric intubation. The rats were killed 1, 5 or 24 hours after dosing and the activity of acetylcholinesterase was measured in both fetal and maternal blood, brain and liver. Toxic signs appeared at the highest dose (2.5 mg/kg) within five minutes. Mortality was high in this group. The activity of acetylcholinesterase was significantly ($P<0.05$) reduced in all maternal and fetal tissue samples taken from surviving dams one hour after treatment with 2.5 mg/kg carbofuran. For lower doses, inhibition was found only in some tissues one hour after treatment. There was a semiquantitative correlation between acetylcholinesterase inhibition in blood and in brain (nervous system). Differential inhibition of isoenzymes of acetylcholinesterase was observed in both maternal and fetal brain (Cambon et al. 1980). This study defines a LOAEL at 0.05 mg/kg for a single dose, based on observed decreases in maternal and fetal blood acetylcholinesterase and maternal liver acetylcholinesterase.

FMC Corporation (1977) conducted a controlled carbofuran exposure experiment using humans. A progression of oral doses beginning with 0.05 mg/kg was established for administration to healthy males (two subjects per dose). The compound was administered as a single dose following breakfast. At the lowest

dose, 0.05 mg/kg, no symptoms were observed. At 0.10 mg/kg, symptoms included headache and a feeling of light-headedness, perhaps unrelated to the treatment. At 0.25 mg/kg, symptoms of acetylcholinesterase depression were observed (dry mouth, salivation, diaphoresis, abdominal pain, drowsiness, dizziness, anxiety and vomiting). As a result, no higher doses were used. The lowest dose used (0.05 mg/kg) can be defined as the NOAEL in this study.

2. Long-Term Exposure

Rotaru et al. (1981) fed male Wistar rats (initial weight 75 g) carbofuran in the diet for 180 days at levels of 0, 10 or 25 mg/kg (ten animals per group). These levels are approximately 0, 0.66 or 1.58 mg/kg/day, calculated using reported final body weights of 213 g and 221 g, respectively, and assuming daily food consumption of 0.014 kg (Arrington 1972). The animals were examined histopathologically after sacrifice and the activity of hepatic enzymes was measured.

Serum enzymes examined included lactic dehydrogenase (LDH), glutamate dehydrogenase, gamma-glutamyl transpeptidase, glutamate oxalate transaminase, glutamate pyruvate transaminase, and alkaline phosphatase. No differences in weight increase or liver weights were observed between controls and treated animals during the experiment. Minor changes observed in liver morphology seemed to relate more to increased caloric intake associated with feeding ad libitum than to the level of carbofuran administered. The only serum enzyme

showing a significant ($P<0.01$) increase was LDH; the increase was observed only at 10 mg/kg (0.66 mg/kg/day) carbofuran. Thus, there appeared to be no dose-response relationship for this effect. This study does not clearly define a NOAEL based on hepatic enzyme effects. There was a significant increase in LDH at 0.66 mg/kg/day, but not at 1.58 mg/kg/day.

Wolfe and Esher (1980) fed old-field mice (Peromyscus polionotus) and cotton mice (P. gossypinus) 100 mg carbofuran/kg food for eight months in order to assess toxic effects. This dose level corresponds to approximately 19.6 mg/kg/day for P. polionotus and 12.2 mg/kg/day for P. gossypinus (based on reported body weights of 13.8 g or 29.5 g and reported daily food consumptions of 2.7 or 3.6 g, for each respective strain). Food consumption, mortality, reproduction and development of young were monitored throughout the duration of the experiment. Male survivors were tested for activity in a behavioral maze. There were no indications of cumulative or delayed effects, except for delayed mortality in P. polionotus. The highest NOAEL that can be defined from this study is 19.6 mg/kg/day.

In a study reported by FMC Corporation (1980d), Charles River CD rats were administered carbofuran at 0, 10, 20 or 100 mg/kg in the diet for two years, equivalent to approximately 0, 0.5, 1.0 or 5 mg/kg/day. Ninety animals per sex per dose were utilized in the study. Parameters observed included body weight, food consumption, behavior, ophthalmoscopy, hematology, biochemistry, urinalysis and histopathology. The only statistically significant change observed was slightly decreased mean body weights for males at

100 mg/kg throughout the study. The 10- and 20-mg/kg groups and all females were comparable to controls throughout the study. There were no effects on cholinesterase at 10 or 20 mg/kg, while at 100 mg/kg, there was some statistically and biologically significant inhibition of plasma, red blood cell and brain cholinesterase. Neoplasms and non-neoplastic histologic changes observed in the animals were judged to be spontaneous lesions unrelated to the exposure to carbofuran during the two-year treatment period. The 20-mg/kg carbofuran diet (1.0 mg/kg/day) is the NOAEL for this study.

In a similar study in mice (FMC Corporation 1980c), Charles River CD-1 mice were fed carbofuran at 0, 20, 125 or 500 mg/kg in the diet, approximately 0, 3, 18.8 or 75 mg/kg bw/day. One hundred animals per sex per dose were utilized in this two-year study. Body weight, food consumption, behavior, hematology, biochemistry, urinalysis and histopathology were observed. The results indicated some effects on body weight at the highest dose (75 mg/kg bw). There was a significant decrease in body weight up to 78 weeks in females and up to 65 weeks in males. By the end of the experiment, however, there were no statistically significant differences in body weights between treated and control animals. Some intermittent reductions in food consumption occurred during the experimental period. At 18.8 and 75 mg/kg bw/day, statistically significant reductions in brain cholinesterase were observed in both males and females; there were no effects at lower doses. No significant effects on plasma or red blood cell cholinesterase were observed at any of dose levels during the experiment.

Analysis of tumor incidence in the animals indicated no treatment-related tumorigenic influence or microscopic changes in organs in any of the carbofuran-treated mice associated with the administration of carbofuran. This study defines a NOAEL of 20 mg/kg food (3.6 mg/kg/day).

In a one-year feeding study in dogs (FMC Corporation 1983), four groups of beagles were administered daily doses of 0, 10, 20 and 500 mg/kg carbofuran in the diet, equivalent to approximately 0, 0.25, 0.50 or 12.5 mg/kg/day, assuming a body weight of 16 kg and daily food consumption of 0.4 kg (Arrington 1972). The animals were allowed access to the treated food for two hours each day. Each group consisted of six males and six females. Of the various biochemical, hematological and clinical observations made, the only positive result was depression of plasma cholinesterase at the two highest dose levels. No biologically significant effects were observed at the 10-mg/kg (0.25 mg/kg/day) level. At 20 mg/kg (0.50 mg/kg/day), there was a consistent, statistically significant depression of plasma cholinesterase in males, but this is not judged to be biologically significant. At 500 mg/kg (12.5 mg/kg/day), there was marked depression of plasma cholinesterase in both males and females and a consistent reduction in erythrocyte cholinesterase. Moreover, there was a treatment-related testicular degeneration with some aspermia in four of the five males at that dose and in one of six males at the 20-mg/kg (0.50 mg/kg/day) dose. In females at the high-dose level, uterine hyperplasia and hydrometria were observed. The conclusion of this study, therefore, is that 20 mg/kg (0.50 mg/kg/day) is the NOAEL for carbofuran in dogs.

B. Quantification of Non-Carcinogenic Effects

1. One-Day Health Advisory

The study by FMC Corporation (1977) has been selected to serve as the basis for calculation of the one-day HAs for children and adults. This study was chosen because it provides an oral, single dose exposure to several levels of carbofuran in humans. The NOAEL observed in this study is 0.05 mg/kg, based on the absence of symptoms of acetylcholinesterase inhibition.

Assumptions regarding absorption are not required in the one-day HA calculations because the NOAEL is expressed in terms of an intake dose rather than an absorbed dose.

The one-day HA for the 10-kg child is calculated as follows:

One-Day Health Advisory for Child:

$$\frac{(0.05 \text{ mg/kg/day})\ (10 \text{ kg})}{(1 \text{ L/day})\ (10)} = 0.05 \text{ mg/L}$$

where:

0.05 mg/kg/day = NOAEL, based on absence of symptoms of acetylcholinesterase inhibition in humans exposed to a single oral dose of carbofuran (FMC Corporation 1977).

10 kg = assumed weight of child.

1 L/day = assumed water consumption by a 10-kg child.

10 = uncertainty factor. This uncertainty factor was selected in accordance with NAS/ODW guidelines for use of a NOAEL from a study in humans.

The same study is used as the basis for the 70-kg adult one-day HA.

The one-day HA for the 70-kg adult is calculated as follows:

One-Day Health Advisory for Adult:

$$\frac{(0.05 \text{ mg/kg/day})\ (70 \text{ kg})}{(2 \text{ L/day})\ (10)} = 0.18 \text{ mg/L}$$

where:

0.05 mg/kg/day = NOAEL, based on absence of symptoms of acetylcholinesterase inhibition in humans exposed to a single oral dose of carbofuran (FMC Corporation 1977).

70 kg = assumed weight of adult.

2 L/day = assumed water consumption by a 70-kg adult.

10 = uncertainty factor. This uncertainty factor was selected in accordance with NAS/ODW guidelines for use of a NOAEL from a study in humans.

2. Ten-Day Health Advisory

No studies involving short-term exposed (one to two weeks) suitable for calculations of a ten-day HA were found in the literature. Because of the rapidly reversible toxic effects of low doses of carbofuran and the absence of evidence of cumulative toxicity, the one-day HAs for children and adults can also serve as the ten-day HA values.

3. Adjusted Acceptable Daily Intake

The one-year feeding study in dogs by FMC Corporation (1983) has been selected to serve as the basis for the ADI and AADI. This chronic study identified 0.50 mg/kg/day as the NOAEL, based on statistically but not biologically significant plasma cholinesterase depression and testicular degeneration in males. Other chronic studies involving rats (FMC Corporation 1980d) and mice (FMC Corporation 1980c) defined NOAELs at higher levels (1.0 and 4.0 mg/kg/day, respectively).

The ADI for a 70-kg adult is calculated as follows:

ADI:

$$\frac{(0.50 \text{ mg/kg/day})\ (70 \text{ kg})}{(100)} = 0.35 \text{ mg/L}$$

where:

0.50 mg/kg/day = NOAEL, based on absence of acetylcholinesterase inhibition and testicular degeneration in beagle dogs exposed to carbofuran via the diet for one year (FMC Corporation 1983).

70 kg = assumed weight of adult.

100 = uncertainty factor. This uncertainty factor was chosen in accordance with NAS/ODW guidelines in which a NOAEL from an animal study is used.

Using 0.35 mg/day as the ADI and assuming the 70-kg adult consumes two liters of drinking water per day, the AADI is calculated as follows:

AADI:

$$\frac{(0.35 \text{ mg/kg/day})}{2 \text{ L/day}} = 0.18 \text{ mg/L}$$

The ADI and AADI derived from the dog study is supported further by evidence from the two-year rat feeding study (FMC 1980d). In this study, groups of rats were fed 0, 10, 20 or 100 mg carbofuran/kg of diet. A NOAEL of 1 mg/kg/day (the middle treatment dose) was identified, to which an uncertainty factor of 200 was applied to protect against cholinesterase depression and systemic effects. This larger than usual uncertainty factor was used in light of the data generated in the single exposure human volunteer

study described above (FMC 1977). Again, it is assumed that the protected individual is a 70-kg adult, consuming two liters of water per day.

This AADI calculation assumes 100% of the human exposure derives from drinking water. This value may be modified upon the availability of relative source contribution data which provide estimates of human exposure from food, air and possibly the occupational environment. Consideration of this relative source contribution to exposure also must lead to a regulatory strategy that yields a long-term health goal that also will be protective of the 10-kg child over shorter durations of exposure.

C. Carcinogenic Effects

The FMC Corporation tested carbofuran for carcinogenicity in both rats (FMC Corporation 1980d) and mice (FMC Corporation 1980c). In the two-year rat study, all neoplasms or non-neoplastic histologic changes observed were judged to be unrelated to the exposure to carbofuran. In the two-year mouse study, the analysis of tumor incidence indicated no treatment-related tumorigenic influence in the carbofuran-treated mice.

A number of short-term assays to assess the mutagenic potential of carbofuran have been performed. In most tests, the results have been negative.

The International Agency for Research on Cancer (IARC) has not evaluated the carcinogenic potential of carbofuran (WHO 1982). Applying the criteria described in EPA's proposed guidelines for assessment of carcinogenic risk (USEPA 1984), the Agency has classified carbofuran in Group E: no evidence of carcinogenicity in humans. This category is used for agents that show no evidence of carcinogenicity in at least two adequate animal tests in different species or in both epidemiologic and animal studies.

D. Quantification of Carcinogenic Effects

Carcinogenic effects are not quantified since there are no data indicating that carbofuran is carcinogenic.

E. Existing Guidelines and Standards

The threshold limit value (TLV) on a time weighted average basis is established at 0.1 mg/m^3 (ACGIH 1980).

NAS (1983) stated that insufficient information exists to permit calculation of a suggested no-adverse-response level (SNARL) or to assess the possibility of chronic exposure hazards. Most toxicological data on carbofuran, however, is classified as Confidential Business Information and was not available to NAS for their evaluation.

F. Special Considerations

The following special considerations are identified for their potential impact on the risk assessment processes.

1. High-Risk Populations

No high-risk populations have been identified.

2. Interactions

Although studies on synergistic effects of methylene dioxyphenyl compounds on carbofuran have not been reported, they might be anticipated by analogy with other carbamate insecticides. No antagonistic effects have been identified.

3. Beneficial Effects

No beneficial effects have been identified.

G. Summary

The recommended values for the one-day and ten-day HAs for both adults and children and the AADI are summarized in Table VIII-1.

Table VIII-1 Summary of Quantification of Toxicological Effects

	Drinking Water Concentration (mg/L)		
	10-kg Child	70-kg Adult	Reference[a]
One-Day Health Advisory	0.05	0.18	FMC Corporation 1977
Ten-Day Health Advisory	0.05	0.18	FMC Corporation 1977
AADI	--[b]	0.18	FMC Corporation 1983

[a] Reference for NOAEL used in calculating HA or AADI values.
[b] Not calculated because the child will not remain in the high-risk population for a lifetime.

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